Conservation Genetics
https://doi.org/10.1007/s10592-019-01199-9

RESEARCH ARTICLE

Current genetic admixture between relictual populations might enhance the recovery of an elusive carnivore

Lise-Marie Pigneur[1] · Gaëlle Caublot[2] · Christine Fournier-Chambrillon[3] · Pascal Fournier[3] · Gloria Giralda-Carrera[4] · Xavier Grémillet[5] · Bruno Le Roux[6] · Daniel Marc[7] · Franck Simonnet[5] · Nathalie Smitz[1,8] · Eric Sourp[9] · Julien Steinmetz[10] · Fermin Urra-Maya[11] · Johan R. Michaux[1,12]

Received: 17 January 2019 / Accepted: 31 May 2019
© Springer Nature B.V. 2019

Abstract

The present study investigated the natural recovery of the Eurasian otter (*Lutra lutra*) in France. The otter was widely distributed in France at the dawn of the 20th century, but then its range considerably shrank and became highly fragmented until the early 1970s, just before it was legally protected. However, for more than 25 years, the otter has been reconquering several parts of its original range and is now considered to be in expansion in France. We investigated the genetic differentiation and diversity of several populations from western and central France and northern Spain to gain insight into the recolonisation dynamics of this elusive species. The present study, based on the use of 14 microsatellite markers, revealed that otter populations seem to be split into five distinct groups. The distribution of samples in those five clusters was closely correlated with suspected refugia where the otter probably survived during the 20th century. Admixture was observed between genetic lineages, possibly enhancing their genetic diversity and thus increasing the recolonisation dynamics of these populations. This phenomenon resembles the genetic pattern noted in many invasive exotic species derived from multiple sources and introduction events. Finally, a demographic approach revealed the probable link between historical human pressure and otter population fragmentation patterns.

Keywords Eurasian otter · France · Genetic diversity · *Lutra lutra* · Population genetic structure · Recolonisation

Introduction

In recent decades, colonisation patterns of poorly genetically diversified populations have been well studied in the biological invasion field. Many recent studies have shown that exotic invasive species might succeed in dispersing in

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10592-019-01199-9) contains supplementary material, which is available to authorized users.

✉ Lise-Marie Pigneur
Lmpigneur@gmail.com

[1] Conservation Genetics Laboratory, University of Liège, Liège, Belgium

[2] Groupe Mammalogique et Herpétologique du Limousin, Aixe-sur-Vienne, France

[3] Groupe de Recherche et d'Etude pour la Gestion de l'Environnement, Uzeste, France

[4] Servicio de Conservación de la Biodiversidad, Gobierno de Navarra, Navarra, Spain

[5] Groupe Mammalogique Breton, Sizun, France

[6] Fédération Aude Claire, Limoux, France

[7] Conservatoire d'Espaces Naturels de Midi-Pyrénées, Toulouse, France

[8] Barcoding Facility for Organisms and Tissues of Policy Concern, Royal Museum for Central Africa, Tervuren, Belgium

[9] Parc National des Pyrénées, Pyrenees, France

[10] Office National de la Chasse et de la Faune Sauvage, Paris, France

[11] Equipo de Biodiversidad, Gestión Ambiental de Navarra, Navarra, Spain

[12] CIRAD, UR ASTRE, Montpellier, France

Published online: 08 June 2019

new areas despite their patchy distribution and low genetic variation (e.g. Tsutsui et al. 2000; Zhang et al. 2010; Pigneur et al. 2014). A similar pattern could perhaps be described in endangered or nearly extinct (or locally extirpated) species that recover and "reconquer" areas in their original range. According to the migrant pool model (Slatkin 1977; Pannell and Charlesworth 1999), individuals of one propagule could derive from several sources in the entire original area. Further admixture of these recolonising populations could then be beneficial for the species' recovery potential. This principle is, for instance, applied in genetic rescue attempts (Frankham 2015; Whiteley et al. 2015). Assessing the genetic diversity of endangered species is indeed of major concern in the conservation biology field since low genetic diversity and inbreeding depression may lead to reduced fitness and lower individuals' adaptation capacity to environmental change (Charlesworth and Charlesworth 1987). We investigated the genetic pattern of a recovering species—the Eurasian otter (*Lutra lutra*)—and described its natural recolonisation dynamics by studying its genetic structure and diversity in western and central France.

The Eurasian otter has been trapped for its flesh and fur since the Middle Ages. Considered as a pest species, this semi-aquatic Mustelid was intensively trapped in France in the 19th century. It was still widely distributed in France and the rest of Europe at the dawn of the 20th century. However, due to a combination of global and local causes (direct persecution, accidental destruction, habitat destruction/degradation or fragmentation, water pollution and consecutive reduction in prey abundance), otter populations were dramatically depleted during the last century (Mathias 1933; Bouchardy 1986; Jefferies 1989; Bouchardy et al. 2011). In France, otter hunting and destruction was banned in 1972. The legal protection of the otter has been reinforced since 1976 (law no 76-629 of 10th july 1976 regarding the protection of nature), but the species has only been strictly protected since 1981 (Kuhn 2011). In 2007, the law was modified to include otter habitat protection (resting and breeding areas). But the otter was already nearly extirpated from most regions in the 1980s. Some relict populations were supposed to have survived along the Atlantic coast and in the Massif Central region (Bouchardy et al. 2011). This could be explained by two main parameters: the human population density and fish prices. In the 19th century, fish price in thermal zones and in large cities was higher than the price of beef. The otter was thus considered an unbearable competitor. In the Pyrenees, the advent of thermal cures was at its height in the 19th century (Penez 2004), leading to a huge pressure on freshwater fishes. The consequence was the persecution of the natural competitors. In these areas, the otter was thus trapped both to avoid competition for fish and for the high commercial value of its skin. The otter population of Pyrenees only maintained on the Spanish side (which was not involved in the spas) and on the marine coast (the fish resource was there mainly exploited in the sea and not in rivers and lakes). On the Atlantic coast, including Brittany, fish resources were mainly linked to the sea while in the rest of the country the otter was considered a pest species due to predation on freshwater fishes. On the Mediterranean littoral, the European eel (*Anguilla anguilla*) was highly sought but largely predated by the otter, leading to an increased competition (Kruuk 2006). Regarding the Massif Central, no large cities were present in regions like Aveyron and Limousin which were troubled by a strong rural exodus (Arbos and Meynier 1936). The correlation between low human densities and advantageous zones (freshwater, food resources, …) has been modelised and it matches with the French strongholds (Reuther and Krekemeyer 2004). This work clearly identifies the Massif Central and Atlantic coast as the most favourable regions for the otter in France.

Otter populations have been gradually recovering since the 1980s, mainly as a result of their legal protection. Marked recolonisation movements were recorded in several regions in the 1990s. The otter had not previously been artificially reintroduced in France, except for an isolated population in Alsace. Reintroductions also occurred in Spain near the French border (e.g. in Catalonia; Saavedra and Sargatal 1997). This endangered carnivore therefore offers an interesting opportunity to assess natural recolonisation movements from refugia. This Mustelid species is currently reoccupying its original distribution area following a severe demographic bottleneck. Moreover, the current genetic structure and diversity of relict populations of this elusive species remain poorly known. Previous studies have shown that, at the European scale, the low mitochondrial DNA diversity found in this species suggests an historical pan-European population (Ferrando et al. 2004; Mucci et al. 2010). Meanwhile microsatellite data have revealed a genetic structure at both global (between European countries/regions) and local (within regions) scales (Randi et al. 2003; Janssens et al. 2008; Mucci et al. 2010; Stanton et al. 2014). In France, the only local detailed genetic survey of otter was conducted in the *Parc Naturel des Cévennes* and two genetic clusters were revealed within this area (Janssens et al. 2008). Moreover, little is known regarding the population density and no relevant population size estimates are currently available (R. Kuhn, pers. comm.).

The present study aimed at investigating otter genetic structure and diversity in a large area that hosted supposed relict populations of western and central France. Samples from northern Spain (Navarra) were included to supplement our sampling of the Pyrenees region (from both sides of the mountain range). We studied several populations through non-invasive sampling and genotyping of 14 autosomal microsatellite loci. The objectives were: i) to determine if

different genetic clusters could be identified in our sample set, and ii) to assess gene flows and admixture levels between them. We also aimed at revealing the demographic history of otter populations and assessing the genetic consequences of the massive declines in otter populations observed in recent centuries in most French regions.

Material and methods

A total of 180 spraint samples were collected in Limousin, Midi-Pyrenees and Massif Central (Lozère) regions in 2013 and 2014 (Fig. 1). We also included 144 samples from dead otters found by chance (mainly road killed individuals) collected mainly between 2000 and 2012 in the Massif Central (Aveyron), Pyrenees, Atlantic coast and Brittany (1988–2014) (Fig. 1). In addition, we included 22 tissue samples from Navarre (Spain), collected also on dead otters between 2003 and 2013. The samples from different periods of time are quite evenly distributed in the studied area and we postulate that the effects on the analyses are limited.

Our tissue sample collection was compiled via collaborators who had the required permits from the relevant national departments. Moreover, the present study was performed as part of the French National Action Plan for European otter conservation, under the direction of the French Ministry of the Environment. No permit was required for feces collection.

Genomic DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN). For faecal samples, we used the QIAmp DNA Stool Mini Kit (Qiagen) in a dedicated laboratory to avoid contamination. Negative controls and aerosol-resistant pipettes were used.

Multilocus genotypes were obtained by PCR amplification of 14 autosomal microsatellites (Dallas and Piertney 1998; Dallas et al. 1999). The forward primer of each locus was 5'-end labeled with a fluorescent dye. The following three multiplex sets were designed: mix 1 (LUT435, LUT701, LUT715, LUT818, LUT833), mix 2 (LUT457, LUT733, LUT782, LUT832) and mix 3 (LUT453, LUT604,

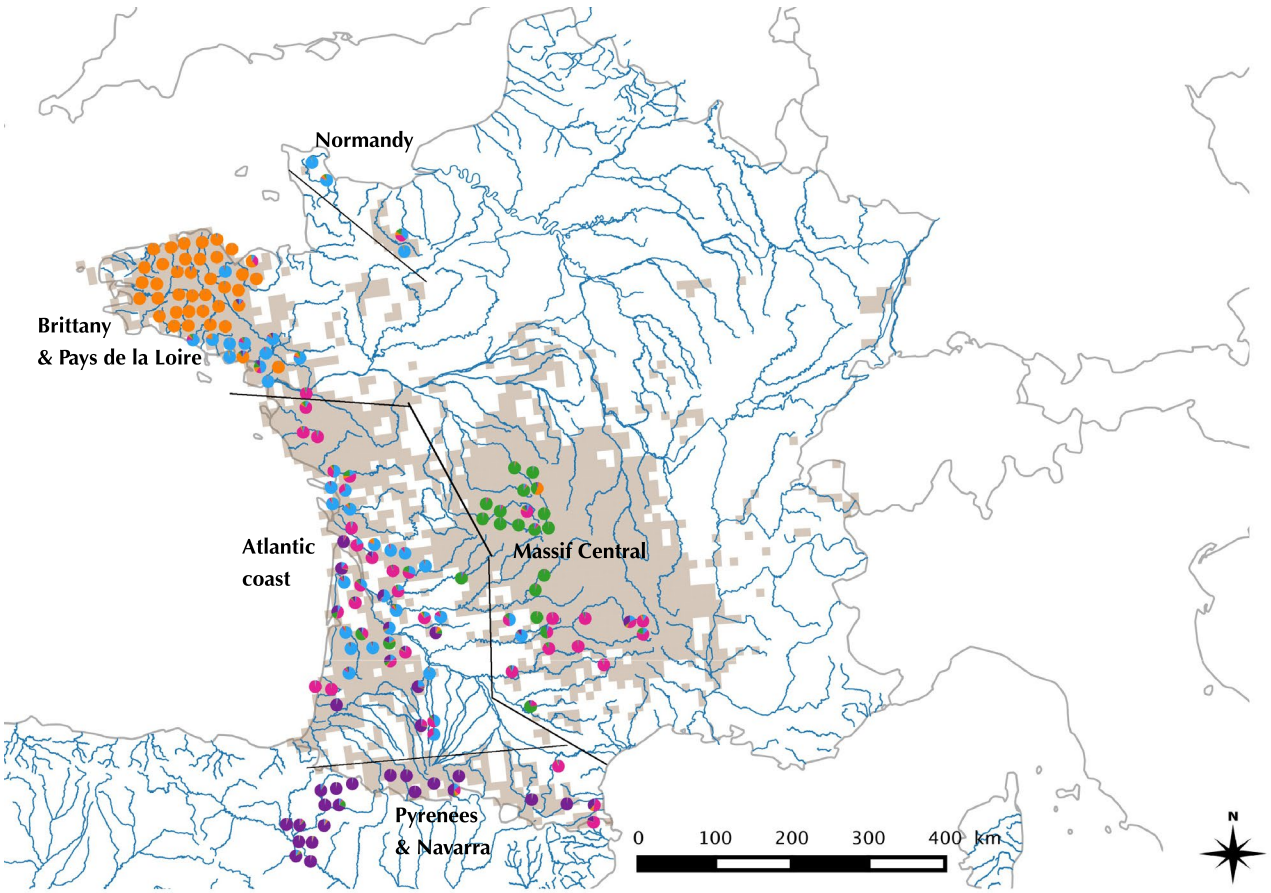


Fig. 1 Map representing the sampled Eurasian otter individuals and their membership coefficient to each of the 5 inferred genetic clusters in western France and northern Spain. The geographic regions defined in the present study are indicated

LUT717, LUT902, LUT914). PCRs were carried out in 10 µl volumes containing 1 µl of primer mix (containing each 2 µM primer), 5 µl of Multiplex PCR Master Mix (QIAGEN) and 1 µl of DNA. For faecal DNA, we used 1.8 µl of primer mix (containing each 2 µM primer), 7.5 µl of Multiplex PCR Master Mix (QIAGEN) and 3.5 µl of DNA, regardless of initial concentration. All amplifications were performed as follows: 95 °C for 15 min followed by 40 cycles (94 °C for 30 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s) and a final extension step at 60 °C for 30 min. PCR products were genotyped on an Applied Biosystems 3130XL Genetic Analyzer using 2 µl of amplified DNA, 10 µl of Hi-Di formamide and 0.15 µl of GeneScan-500 (LIZ) size standard (Applied Biosystems). Length variation determination (alleles and genotypes) was performed using GENEMAPPER 4.0 (Applied Biosystems). MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to detect stutter errors and to estimate the proportion of null alleles at each locus for each cluster defined by the preliminary STRUCTURE analysis (see below). Genotypes were then corrected accordingly.

For spraint samples, we used a multitube approach to avoid allelic dropout and false alleles by repeating amplifications 4 to 6 times. To construct consensus multilocus genotypes, we followed the rules described hereafter (adapted from Bonesi et al. 2013): an allele was only accepted if observed at least twice. We thus accepted heterozygous genotypes that were observed twice. A homozygote was accepted after three positive PCRs gave the same single allele; otherwise we coded the locus as being potentially heterozygous with only one allele identified and one allele missing. Individual identification of genotypes from faecal samples was performed using GIMLET (Valière 2002), 'Regroup genotypes' option.

We used GENEPOP (http://genepop.curtin.edu.au/; Raymond and Rousset 1995) to perform tests for linkage disequilibrium between loci for each genetic cluster or each geographic population (see below) with the following parameters: 1000 dememorizations, 1000 batches and 1000 iterations per batch. The significance level was then adjusted following Bonferroni correction for multiple tests (Rice 1989).

The genetic structure of the otter populations was inferred using Bayesian clustering analysis with STRUCTURE 2.3 software (Pritchard et al. 2000). We ran 10 iterations for each K value from 1 to 10 using the admixture model. A total of 10^6 MCMC repetitions were performed after a burn-in period of 10^5. The results of the 10 iterations for each K value were summarized and averaged using the CLUMPP method (Jakobsson and Rosenberg 2007) implemented in CLUMPAK (Kopelman et al. 2015). The optimal number of clusters was investigated using the increasing likelihood of the data (Pritchard et al. 2000) as well as the ΔK method (Evanno et al. 2005), implemented in STRUCTURE HARVESTER web version 0.6.94 (Earl and vonHoldt 2012). The distribution map of the studied individuals and their cluster membership was designed under QGIS version 1.8.0 Lisboa (Quantum GIS Development Team 2013). Open Source Geospatial Foundation Project. http://qgis.osgeo.org). On the one hand the ΔK method (Evanno et al. 2005) is likely to identify the uppermost level of hierarchical population structure, leading to underestimating of structure. On the other hand, uneven sample size may affect the recovery of population structure in data sets (Puechmaille 2016). We therefore used StructureSelector (Li and Liu 2018) to validate the results. Admixture between geographic populations was also estimated using another modeling analysis using GeneClass2 (Piry et al. 2004). This analysis estimates putative first generation migrants ($p<0.05$) and putative origin populations. We used the option « detection of first generation migrants » with the frequencies-based method (Paetkau et al. 1995) and an assignment thresshold of 0.05 for the likelihood computations (option « L = L_home/L-max »).

In addition, we performed a discriminant analysis of principal components (DAPC) (Jombart et al. 2010). This multivariate analysis is not based on population genetics models and is robust to deviations from Hardy-Weinberg equilibrium and linkage equilibrium. We implemented DAPC in *adegenet* version 1.3–8 (Jombart and Ahmed 2011) in R version 2.15.2 (R Development Core Team 2008) to identify and describe clusters of genetically similar individuals. The most likely number of genetic clusters was determined using the k-means clustering algorithm (Legendre and Legendre 1998), for $K = 1$ to $K = 20$, via the *find.clusters* function, with all principal components (PCs). The appropriate number of clusters was defined using the Bayesian information criterion (BIC) through the distribution of BICs corresponding to all possible clusterings and with the lowest value being generally indicative of the best clustering. The *optim.a.score* function was used to determine the optimal number of PCs and a final DAPC was carried out with this optimal number.

For subsequent analyses, individuals were sorted in different ways: (i) according to their geographic origin (sorted into 4 main regions: Brittany and Pays de La Loire, Atlantic Coast, Massif Central, Pyrenees and Navarra), and (ii) sorted in 5 sub-populations according to the STRUCTURE results (admixed individuals ($q < 0.9$) were excluded).

F-statistics (pairwise F_{ST}, D_{Jost} and Fis), allelic richness (Ar) and the expected (H_e) and observed (H_o) heterozygosity were calculated for each defined group using *diveRsity* (Keenan et al. 2013) in R version 2.15.2 (R Development Core Team 2008).

Neighbour-joining (NJ) trees were built with POPULATIONS 1.2.32 software (Langella 2007) to represent relationships between genetic clusters (results not shown; the

trees are represented in the demographic scenarios of Fig. S1). We used D_A (Nei et al. 1983) and Ds distance, and F_{ST} to obtain the tree topologies (Takezaki and Nei 1996). The robustness of nodes was evaluated by bootstrapping on populations (1000 replicates). The obtained trees were used to select and design competing scenarios for the demographic Bayesian approach. Ten plausible demographic scenarios of the evolutionary history of the population were designed according to the observed genetic structure and the phylogenetic trees (Fig. 2). All ten scenarios were tested in one run. Approximate Bayesian computation (ABC) analyses are valuable tools to investigate complex population genetics patterns based on microsatellite markers (e.g. Bryja et al. 2010; Sunnaker et al. 2013; Mondol et al. 2013; Ali et al. 2014; Olafsson et al. 2014). These analyses were performed using coalescent-based software DIYABC 2.0.4 (Cornuet et al. 2014). They allowed us to determine potential relations among different clusters, interpreted thereafter as populations, and to estimate the timing of their differentiation. The range and distribution of priors for parameters used to describe the scenarios (effective population size, time of events, model parameters) are presented in Table S1. We used microsatellite mutation rates generally used for mammals (i.e., 10^{-3} to 10^{-5}; Dallas 1992; Weber and Wong 1993; Ellegren 1995) and broad intervals for each parameter, so that uncertainties regarding these parameters would not affect the output of the analysis. To estimate the time of differentiation events, we considered a minimum generation time of 2 years, as generally considered for this species (Koelewijn et al. 2010; Kuhn 2011). A total of 1.000.000 datasets was simulated for each scenario by building a reference table from a specified set of prior parameter distributions. A principal component analysis (PCA) was performed on the first 10,000 simulated datasets to check whether the set of scenarios and the prior distributions of their parameters could generate datasets similar to that observed (Fig. S1). A normalized Euclidean distance between each simulated dataset of the reference table and the observed dataset was calculated to identify the most likely scenario. To estimate the relative posterior probability (PP) of each scenario, 1% of the closest simulated datasets was used in a logistic regression. The scenario harboring the highest posterior probability was considered the most likely.

We estimated the type-I and type-II error according to Cornuet et al. (2010) by simulating 500 pseudo-observed datasets displaying properties similar to those of the observed dataset under the best scenario and computed the proportion of cases in which this scenario did not display the highest posterior probability among all scenarios (Table S2). We estimated the false positive error rate (type-II error) by simulating 500 pseudo-observed datasets under each alternative scenario and computed the proportion of cases in which the scenario with the highest PP was incorrectly selected as the most likely scenario.

Results

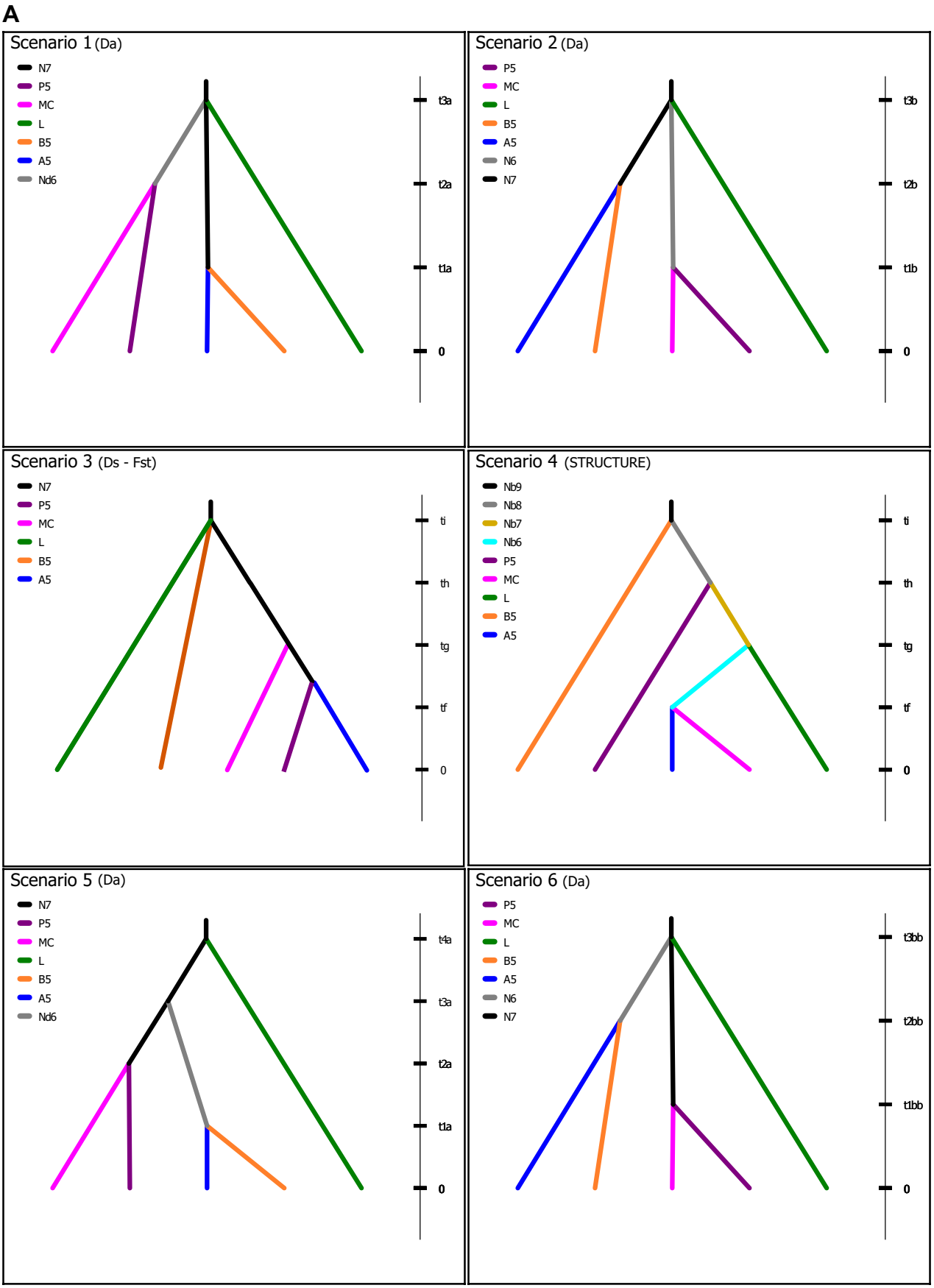
Individual identification from faecal DNA

A total of 41 genotypes were derived from 180 spraint samples. The analysis performed using GIMLET suggested that these 41 genotypes belonged to a maximum of 39 distinct individuals (however missing data within genotypes may prevent genotype regrouping). Thus, in two cases, the same genotype was found in two distinct spraint samples (found in the same region). The final analyses only included the 39 distinct genotypes.

Global genetic structure

A total of 205 distinct multilocus genotypes (39 from faecal samples and 166 from tissues) was obtained. According to the STRUCTURE results, 3 to 5 genetic clusters should have been considered in our dataset (highest ΔK value for $K = 3$ and best $K = 5$ according to Ln P(D); Fig. S2). Under the $K = 3$ hypothesis, one cluster was exclusively found in Brittany region (called cluster B3 hereafter) (except for one sample found in Limousin, Massif Central). The second cluster (A3 hereafter) was widespread and found in the Massif Central, Atlantic coast and Pyrenees regions. Finally, the third cluster (P3 hereafter) was mostly found in the Pyrenees (but also in the Atlantic region). The second hypothesis, $K = 5$, was jointly supported by the LnP(D) values and k-means algorithm results (lowest value for BIC at $K = 5$; Fig. S3). Under this hypothesis, 74 out of 205 genotypes (36%) had an admixed pattern ($q < 0.9$) according to STRUCTURE analysis. Specific clusters were again found in Brittany and Pyrenees regions (clusters B5 and P5 hereafter). A new cluster was found in the Massif Central, mostly in the Limousin region (cluster L hereafter) and a second one in the southern part of the Massif Central (Lozère and Aveyron), (MC hereafter). Finally the last cluster was spread mostly along the Atlantic coast (cluster A5 hereafter) (Fig. 3). The DAPC suggested the same geographic pattern, with Brittany and Pyrenees more isolated from the others (Fig. 4). Analysis with StructureSelector retrieved the uppermost structure with Brittany population being the most distinct group (results not shown).

To further investigate the admixture between populations, we used GeneClass2 (Piry et al. 2004). The simulations detected 19% of putative migrants (results not shown) and the samples assignment was highly congruent with the results of STRUCTURE analysis.


A
Scenario 1 (Da)
N7
P5
MC
L
B5
A5
Nd6
t3a
t2a
t1a
0
Scenario 2 (Da)
P5
MC
L
B5
A5
N6
N7
t3b
t2b
t1b
0
Scenario 3 (Ds - Fst)
N7
P5
MC
L
B5
A5
ti
th
tg
tf
0
Scenario 4 (STRUCTURE)
Nb9
Nb8
Nb7
Nb6
P5
MC
L
B5
A5
ti
th
tg
tf
0
Scenario 5 (Da)
N7
P5
MC
L
B5
A5
Nd6
t4a
t3a
t2a
t1a
0
Scenario 6 (Da)
P5
MC
L
B5
A5
N6
N7
t3bb
t2bb
t1bb
0

◀ **Fig. 2** Representation of 10 competing scenarios tested with approximate bayesian computation for Eurasian otter populations in western France and northern Spain. This analysis was based on the five genetic clusters according to STRUCTURE analysis assignment (excluding individuals with $q < 0.9$). N_i corresponds to the effective population size of each cluster, and t_i corresponds to the time expressed in numbers of generations since divergence. Abbreviations of cluster names are as follows: *P5* Pyrenees, *MC* Massif Central, *L* Limousin, *B5* Brittany, *A5* Atlantic, *PP* posterior probability and associated 95% confidence interval

For the subsequent analyses, samples were either sorted according to the STRUCTURE clusters (5) or according to their geographic origin sorted in 4 main regions: Brittany (including Pays de la Loire), Massif Central, Pyrenees (including Navarra) and Atlantic regions (due to their low number, the four individuals from Normandy were discarded from this analysis).

Genetic diversity

Genetic diversity estimates obtained through the analysis of 14 microsatellites in 207 otter samples (205 genotypes) are shown in Tables 1 and 2. All loci were polymorphic in all sampled populations/clusters.

For the analysis based on geographic populations, the average allele number per locus was 4.9 and ranged from 4 (Brittany region; A = 4.2) to 6 alleles (Atlantic region; A = 5.8). The mean allelic richness per locus ranged from 2.5 (Brittany region) to 2.8 (Atlantic region). For the genetic clusters (K = 5), the average allele number per locus was 3.7

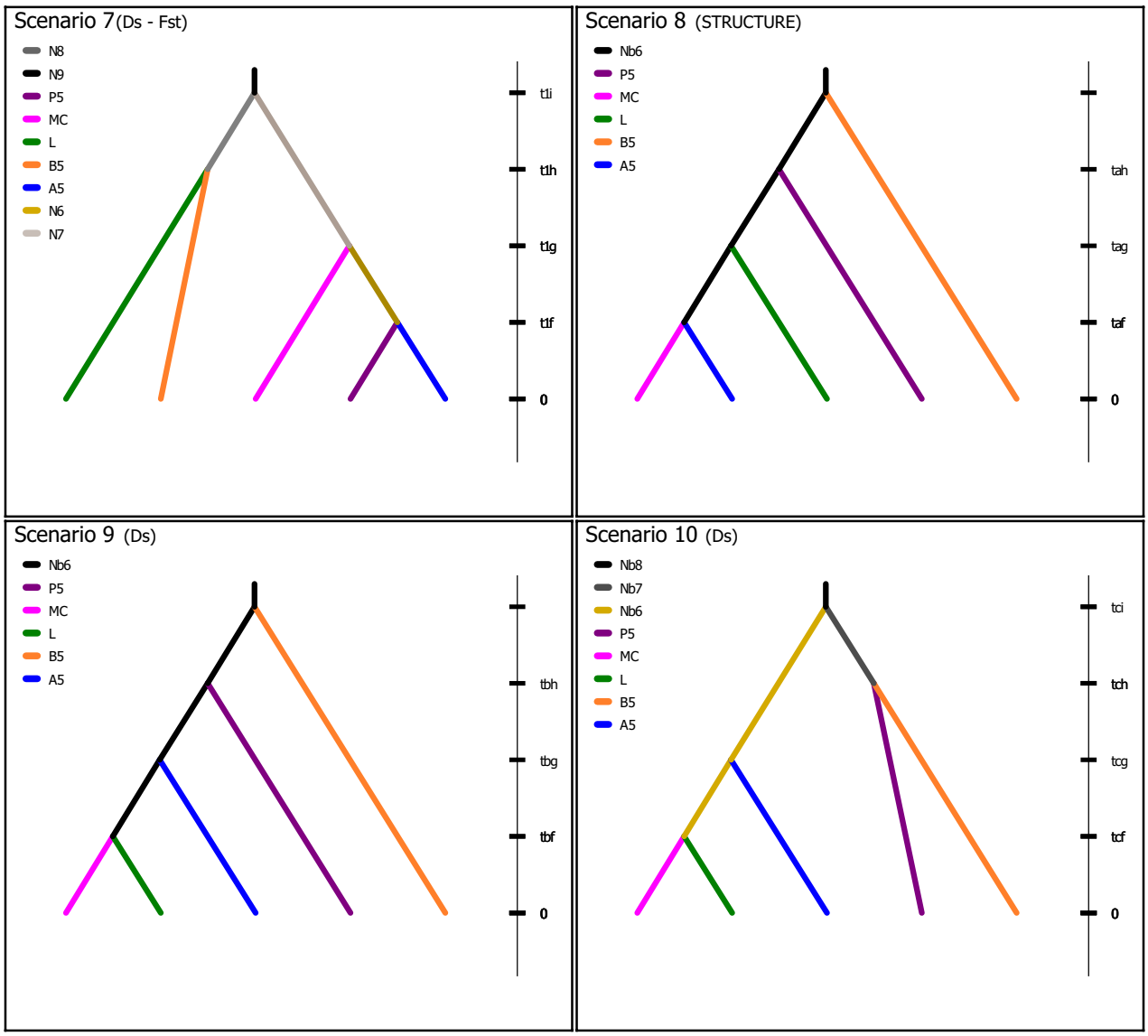


Fig. 2 (continued)

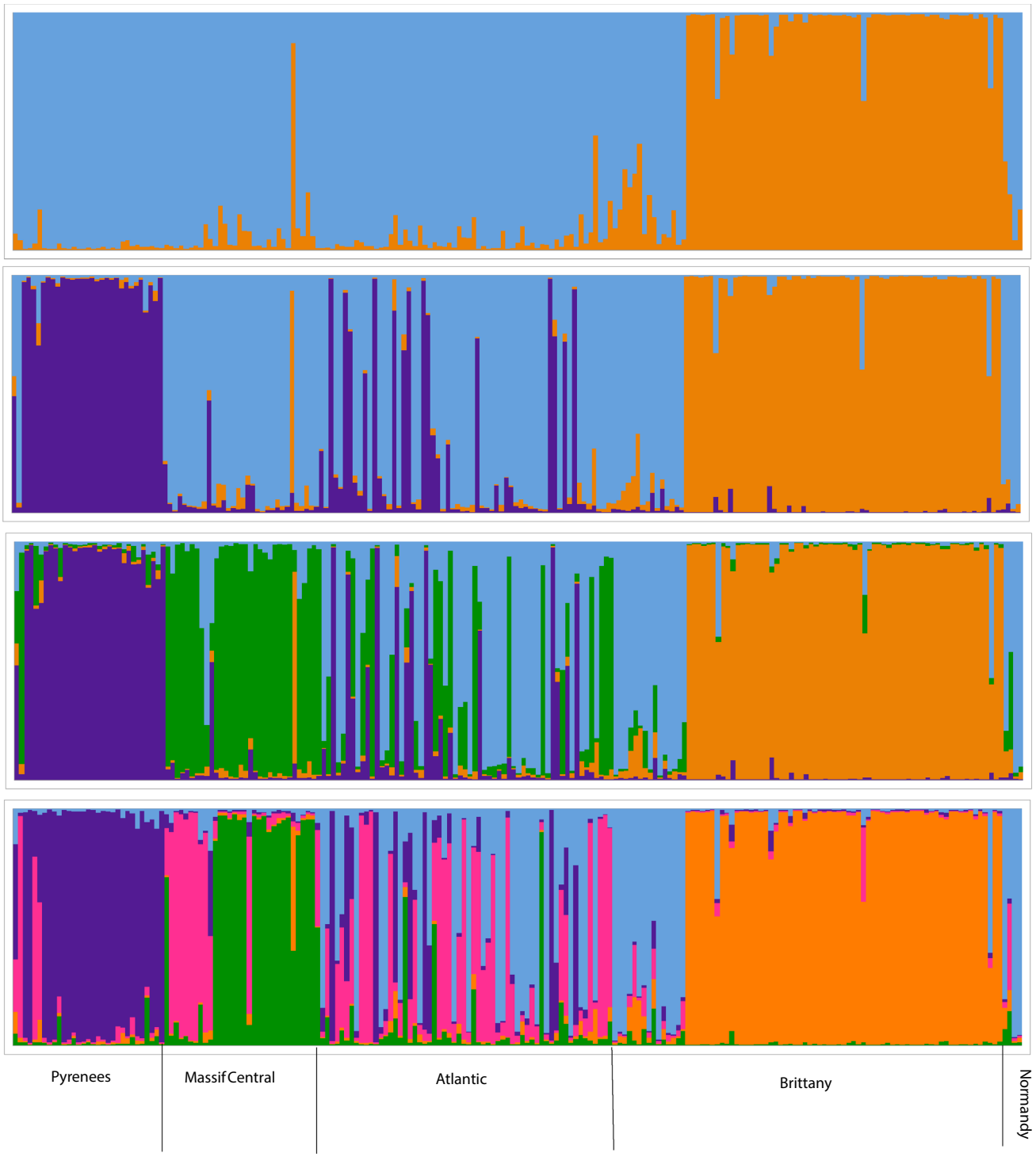


Fig. 3 Barplot of membership coefficients according to the STRUCTURE results ($K = 2$ to $K = 5$) based on microsatellite loci for the Eurasian otter in western France and northern Spain

and ranged from 3 (cluster B5; A = 2.9) to 4 (cluster MC; A = 3.8) alleles. The mean allelic richness per locus ranged from 2.5 (B5) to 3.7 (A5).

No locus was found to show significant linkage disequilibrium in any population after Bonferroni's correction. Hardy-Weinberg exact tests performed on each cluster at each locus showed no deviation from the expected frequencies after Bonferroni's correction.

The F_{ST} and D_{Jost} estimates are presented in Table 1. The highest pairwise values (exceeding 0.2) were recorded

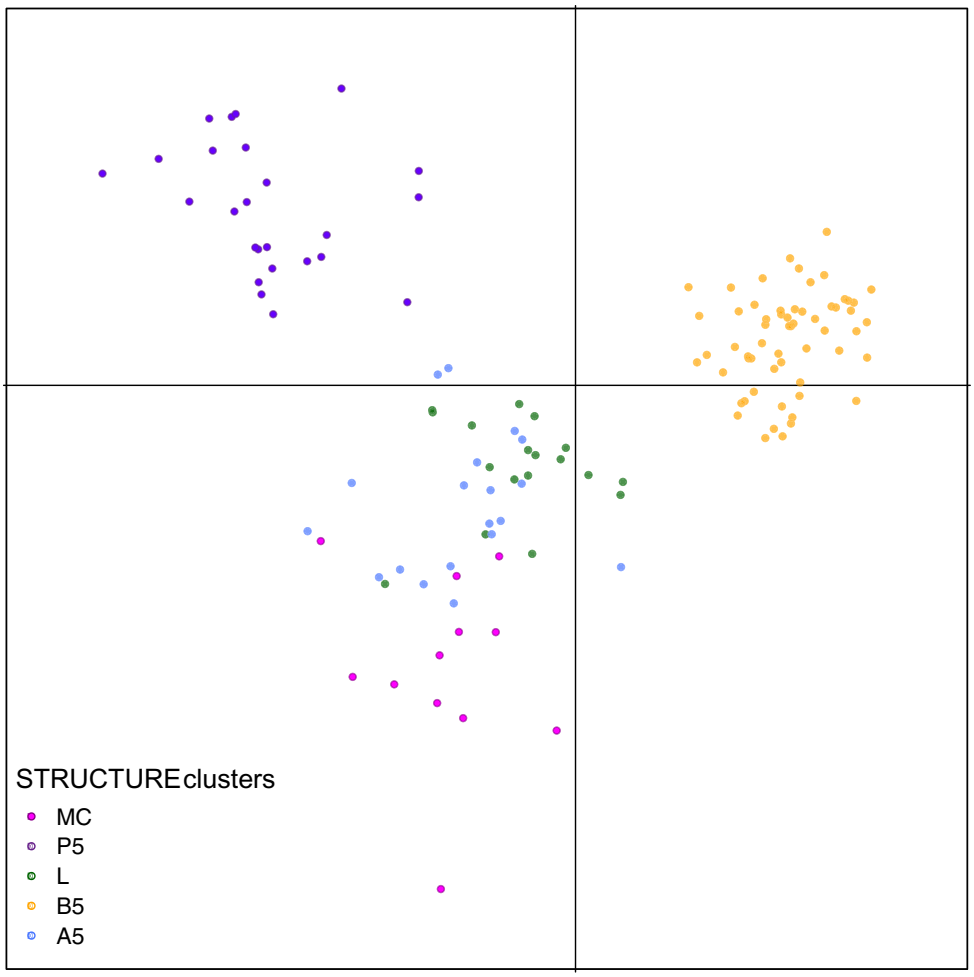


Fig. 4 Discriminant analysis of principal components plot showing the positions of five clusters (determined by k-means algorithm) of Eurasian otters in western France and northern Spain. Colors correspond to similar clusters determined by STRUCTURE in Fig. 3. (*MC* genetic cluster mainly found in Massif Central for $K = 5$: P5 = Pyrenees, MC = Massif Central, L = Limousin, B5 = Brittany, A5 = Atlantic)

between clusters B5 and P5 and between B5 and MC (D_{Jost} estimates) and between B5 and each of the other clusters (F_{ST} estimates). The F_{ST} values indicated that the lowest gene flow was recorded between B5 and all the other ones, and between L and P5 clusters. B5 had the highest F_{IS} values although the estimate was not significantly higher than in the other clusters. This cluster also had the lowest allelic richness (Table 2). The pairwise F_{ST} and D_{Jost} indexes supported the highest genetic exchanges between L and neighbouring MC. (Table 2). Regarding the geographic populations, the highest pairwise values (F_{ST} and D_{Jost}) were recorded between Brittany and Pyrenees. The lowest pairwise values were recorded between Massif Central region and the Atlantic Coast (Table 2).

Demographic history

The analysis was performed on the five inferred genetic clusters in order to determine potential relations among them and to estimate the timing of their differentiation. The PCA performed on the first 10.000 simulated datasets revealed that the observed dataset was surrounded by simulated datasets. Our model was thus able to produce datasets similar to that observed (Fig. S1). Ten plausible demographic scenarios of the evolutionary history of the five inferred genetic clusters (Fig. 2) were tested using DIY ABC 2.0.4 (Cornuet et al. 2014). The logistic regression carried out on the 1% simulated datasets closest to the observed one revealed that Scenario (SC) 5 had the highest posterior probability (PP = 0.2814, 95 CI [0.2106–0.3523]). However, its 95% confidence interval overlapped that of SC1 (PP = 0.2053, 95 CI [0.1527–2579]; Table 3) which depicted a very similar pattern (Fig. 2). We estimated the performance of the analysis and out of the thousands of pseudo-observed datasets generated using SC5, 42.2% were correctly identified as having been generated under this model. A moderate proportion (18.6%) was wrongly attributed to SC 1. The Type-II error rate was low considering datasets generated under most scenarios, and up to 14.8% with SC1 (Table S2). The performance analysis also suggested that SC5 could be confounded with SC1.

In SC5 (Fig. 2), all observed clusters originated from a common, unsampled ancestral population (N7) that gradually became fragmented over time in several distinct

Table 1 Pairwise FST (above diagonal) and Jost's D (below diagonal) estimates calculated for: (a) the five genetic clusters, and (b) the geographic populations of Eurasian otter in western and central France

(a)	P5	MC	L	B5	A5	(b)	Pyrenees	Massif Central	Brittany	Atlantic Coast
P5	–	0.1658 [0.1224–0.215]	0.1951 [0.1563–0.2436]	0.3009 [0.2684–0.3342]	0.1818 [0.1475–0.2218]	Pyrenees	–	0.1064 [0.0699–0.1427]	0.2037 [0.1694–0.2438]	0.0706 [0.0454–0.0984]
MC	0.1661 [0.0963–0.2513]	–	0.1233 [0.076–0.1913]	0.2327 [0.1907–0.2833]	0.1408 [0.1011–0.1921]	Massif Central	0.1024 [0.0576–0.1538]	-	0.1397 [0.1101–0.1715]	0.0522 [0.0273–0.0816]
L	0.1827 [0.1323–0.2396]	0.0982 [0.0395–0.1818]	–	0.2318 [0.2012–0.2664]	0.194 [0.1552–0.2383]	Brittany	0.1932 [0.15–0.2395]	0.1191 [0.0826–0.1602]	–	0.1368 [0.1093–0.1655]
B5	0.2913 [0.2398–0.3462]	0.2217 [0.1582–0.3035]	0.1828 [0.1423–0.2359]	–	0.2335 [0.2059–0.2606]	Atlantic Coast	0.0595 [0.0336–0.0937]	0.0357 [0.0093–0.0657]	0.1269 [0.0971–0.1606]	–
A5	0.1501 [0.1061–0.2003]	0.1455 [0.0869–0.215]	0.1439 [0.0988–0.1939]	0.1821 [0.139–0.2303]	-					

Table 2 Genetic diversity at the 14 microsatellite loci for: (a) the five genetic clusters and (b) the geographic populations of Eurasian otter in western and central France

	N	F_{IS} [CI]	Ar	Ho/He
(a)				
A5	19	−0.0835 [−0.1764 to 0.003]	3.16	0.56/0.52
B5	59	0.0600 [−0.0098 to 0.1324]	2.47	0.45/0.48
L	18	−0.0683 [−0.1784 to 0.1324]	3.01	0.54/0.5
MC	12	0.0500 [0.1375 to 0.2111]	3.70	0.59/0.62
P5	25	0.0451 [−0.05 to 0.1385]	3.54	0.52/0.55
(b)				
Atlantic	59	0.0849 [0.0155 to 0.1522]	2.77	0.56/0.61
Brittany	82	0.0946 [0.0421 to 0.1523]	2.45	0.48/0.54
Massif Central	30	−0.0319 [−0.1066 to 0.0407]	2.58	0.61/0.59
Pyrenees	36	0.0568 [−0.0214 to 0.1408]	2.72	0.54/0.58

N sample sizes, *FIS* inbreeding coefficient, *Ar* mean allelic richness, *CI* confidence intervals, *Ho* observed heterozygosity, *He* expected heterozygosity

groups (Fig. 5). More recently, the last two subgroups split up, resulting in the five observed clusters. The median (95% CI) of the estimated time since divergence (t_1) between clusters B5 and A5 was evaluated at about 95 generations earlier, and 157 generations earlier for P5 and MC. Assuming a minimum generation time of 2 years (Kuhn 2011), these divergence times would correspond to at least 190 and 314 years ago, respectively. The effective population size estimates for the clusters ranged from 141 (B5) to 935 (MC) individuals (Fig. 5). There was, however, a lack of sufficient field data to determine the ratio between the census population size and the effective population size of otters in France.

Discussion

In the early 20th century, the Eurasian otter likely had a Pan-European distribution (Pérez-Haro et al. 2005; Mucci et al. 2010). The present study suggests the existence of three to five genetic clusters in the studied populations from western France and northern Spain, with a clear geographic pattern. The genetic differences detected between most regions likely reflected the population fragmentation that occurred during the last centuries. The strongholds where the species survived seem to have been located in these regions. The original population seems to have started fragmenting several centuries ago according to the demographic simulations. Caution is nevertheless needed when considering these estimates since demographic ABC inferences are particularly sensitive to the priors used in the analysis (Demenou et al.

Table 3 Posterior Probabilities (PP) of competing demographic scenarios and corresponding confidence intervals (CI)

	SC 1	SC 2	SC 3	SC 4	SC 5	SC 6	SC 7	SC 8	SC 9	SC 10
PP	0.2053	0.0407	0.0224	0.0738	0.2814	0.1046	0.0239	0.0785	0.0919	0.0775
CI	[0.1527–0.2579]	[0.0025–0.0790]	[0.0000–0.0633]	[0.0336–0.1140]	[0.2106–0.3523]	[0.0681–0.1410]	[0.0000–0.0648]	[0.0404–0.1165]	[0.0477–0.1361]	[0.0359–0.1190]

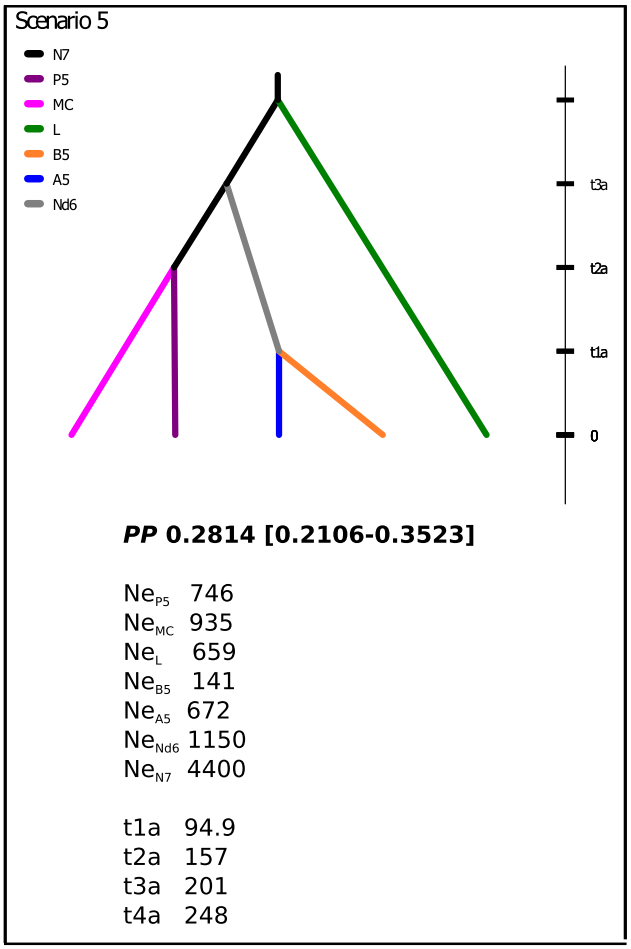


Fig. 5 Representation of demographic scenario 5 and parameter estimates for Eurasian otter populations in western France and northern Spain. (*MC* = genetic cluster mainly found in Massif Central for *K* = 5, P5, L, B5, A5=)

2016). In order to decrease this risk, priors were chosen very carefully and we used microsatellite mutation rates generally used for mammals (i.e., 10^{-3} to 10^{-5}; Dallas 1992; Weber and Wong 1993; Ellegren 1995).

Although these relatively distant split times were only rough estimates, they however suggested that historical pressures on otters might have been higher than previously thought and the recent persecutions and pollution in the 20th century might then have precipitated the massive decline observed 60 years ago. In the second half of 20th century, after its legal protection, the recovering otter populations may have been able to re-expand and colonise neighbouring regions from these putative refugia. The Brittany population seemed to be more isolated and exhibited lower genetic diversity. This might have been the result of a strong bottleneck in this region, after its isolation from the other clusters or it could instead have been due to lower effective population size (Ne) compared to the other refuge populations.

Indeed, in the early 1980s, the population of Central Brittany population was restricted to a very limited number of rivers in a small area.

Although distinct genetic clusters were recorded, they seem to have recently met and started to admix. There is now an ongoing process of reconnection of these populations. On the one hand, we found different genetic clusters within most regions. On the second hand, gene flow was observed between most regions and clusters and a high proportion of admixed individuals was recorded (35.7% for $K = 5$). We postulate that there was an increase in genetic diversity following the admixture. However the genetic diversity level before the admixture is unknown and it is therefore uneasy to assess how it changed. The current genetic diversity is still moderate (maximum mean allelic richness of 3.7) in comparison with that found for example in Britain (mean allele number per locus ranging from 2.2 to 5.3; Dallas et al. 2002). Finally, the dispersal of some individuals far away from the geographic "epicentre" of their genetic cluster (and corresponding refugia; Fig. 1) is evidence of the high dispersal potential of the otter and thus the possibility of admixture between the observed clusters. This ongoing admixture could be beneficial for the species by reinforcing its genetic diversity.

Evolutionary perspective

Low genetic diversity in endangered species may result from recent pressures but can also be of ancestral/historical origin (Matocq and Villablanca 2001). This has to be taken into account in management plans. In the present study, the genetic structure seemed to be linked to relatively recent events (maximum 500 years ago). The observed genetic clusters would have derived from modern "refugia" where the species survived over the last centuries, rather than reflecting ancient post-glacial refuges. Indeed, in the 1980s, the last traces of the species were recorded in Brittany, Pyrenees, Massif Central and along the Atlantic coast in Landes and Gironde departments (Green and Green 1981). Moreover, previous studies (Mucci et al. 2010) evidenced, that at the European scale, this species probably survived during the last ice age in a single refuge population, from which it recolonised the whole continent. Therefore, the structure we observed in our study would not be explained by the survival of the otter in different glacial refuges.

The story of the recovery of the Eurasian otter in France supports the species and ecosystem resilience concept. This phenomenon is, for example, well documented for haplochromine cichlids in Lake Victoria. After the introduction of the Nile perch (*Lates niloticus*) in the 1950s and 1960s, the amazing diversity of small cichlids had nearly disappeared (Ogutu-Ohwayo 1990). However, recent studies have revealed that several haplochromine species are now recovering, probably linked with the recent decline of Nile perch (Witte et al. 2000; Awiti 2011).

The evolutionary potential is reduced when genetic diversity is already low, but genetic diversity can be increased by new exchanges when genetically differentiated populations are admixed. This happens, among others, in invasive species with multiple origins and repeated introductions (e.g. Lavergne and Molofsky 2007; Kolbe et al. 2004). Here we described a similar pattern in recovering populations of an endangered species. The otter recovery trend is quite exceptional and to date there is no comparable example in other European mammals. The observed level of genetic diversity was in the same range as that observed by Mucci et al. (2010), with an average allelic number A of 4.9. The current admixture events observed in French otter populations have helped reinforce the genetic diversity and the subsequent recovery process. This genetic reinforcement is probably enhanced by the fact that the genetic diversity observed in most clusters (except for B3/B5) was not markedly reduced and that the F_{IS} estimates were not particularly critical. This is quite surprising for a species that underwent massive persecutions. The otter therefore seems to have survived more successfully in refugia (thus retaining more genetic diversity) than previously thought, or its population size before the persecutions was possibly particularly high to maintain a good level of genetic diversity despite these human impacts. However, there are no precise estimates of the overall otter population size before and after persecutions (Kuhn R., pers. com.). The present study offers estimates of Ne for the different genetic clusters, but these values might be significantly lower than the census population size (N). Among others, Ne is particularly sensitive to population size fluctuations, unequal numbers of males and females and spatial dispersion patterns. It is difficult to correctly link N and Ne, especially in elusive and nocturnal species for which it is hard to conduct a population census (Palstra and Fraser 2012). Koelewijn et al. (2010) found that Ne was around 30% of the observed density (N) in the reintroduced otter population in the Netherlands. Moreover, demographic ABC inferences are particularly sensitive to user-defined priors, which may largely influence posterior parameter estimates (Demenou et al. 2016).

For cluster L, mainly found in Limousin region, our Ne estimate reached 659 individuals while exhaustive field records have lead to estimates of census population sizes ranging from 1500 to 2800 individuals (Leblanc et al. 2005). However, these estimates were theoretical and based on the hypothesis of full and optimal water system occupation.

Although the recolonisation process is under way, the future of the studied otter population is still uncertain and fragile. It is noteworthy that the areas where the species survived over the last century have low human densities and high food resources. Nowadays, although the otter

distribution is expanding, it is mainly restricted to French regions that are the most favourable to this species (regarding habitat, resources, etc.), as also observed in other European countries (Kuhn 2011). In the Midi-Pyrenees region, where the habitat is advantageous for otters, the colonisation rate was estimated at 27% over a 10-year period (Steinmetz et al. 2014). However, it is still difficult to estimate whether this species will, in the long run, be able to recolonise other regions where human densities and activities are higher or whether these regions will only serve as dispersal corridors.

Implications for conservation

The case of the French otter populations is a striking example of natural recovery in mammals. Indeed, this species is currently in a recolonisation phase in most European regions (Stanton et al. 2014; Janssens et al. 2008). The role of hydrologic continuity and riparian corridors for the otter has already been highlighted in Central France (Bouchardy et al. 2011). Moreover, this species is characterised by a high dispersal potential, as demonstrated by the post-glacial recolonisation of Europe probably from a single refuge population (Mucci et al. 2010). Several surveys have also underlined the high mobility of this carnivore and its ability to cross many putative barriers (Janssens et al. 2008; Leblanc et al. 2005). The enhanced genetic diversity that might arise from the current genetic admixture might be beneficial for the future of the Eurasian otter in France. Admixture can sometimes have detrimental effects on fitness if populations previously had high degrees of local adaptation (Edmands 2007). However, in the present case, the risk of outbreeding depression seems negligible because of the historical connectivity and the obvious lack of adaptive divergence (Frankham et al. 2011).

The European badger (*Meles meles*) is another Mustelid that has been able to recover thanks to protection measures, including in The Netherlands (Griffiths and Thomas 1997). In Europe, several large carnivores are also recovering or persisting in human-dominated landscapes (Chapron et al. 2014). The pattern observed for the otter in France is probably similar to that of the grey wolf (*Canis lupus*) and the Eurasian lynx (*Lynx lynx*) in Central and Western Europe although their larger size and higher dispersal capacities (combined with sufficient genetic diversity) are most likely to contribute to their recolonisation success.

The otter seems to exhibit a particularly high recolonisation potential. Its high mobility, associated with the multiple refugia where the species has managed to survive in different French regions, has resulted in this specific pattern, i.e. quite high genetic diversity compared to other mammals with declining population sizes in the 20th century. This contrasts with the case of other threatened mammals such as the Iberian lynx (*Lynx pardinus*) (Casas-Marce et al. 2013) or the European mink (*Mustela lutreola*), both probably suffering among others from low genetic diversity resulting in a reduced adaptative potential. The European mink is a riparian Mustelid that occupies the same habitats as the otter but it is nowadays critically endangered (Maran et al. 2016). Nevertheless the causes of the disappearance of both species are not fully comparable. The European mink still suffers from several pressures; it can be trapped by confusion with other Mustelids, and it is also impacted by the American mink (*Neovison vison*) which is colonising its range. While reasonably high genetic diversity was observed in most otter clusters (except Brittany), extremely low genetic diversity has been observed in European mink in France (all Western European populations were characterised by one single mitochondrial haplotype and a mean allelic richness of 2 for all analysed microsatellites), which could lead to an inbreeding depression phenomenon (Michaux et al. 2005; Cabria et al. 2015).

The genetic diversity level is of considerable concern for conservation biologists. Indeed, reduced genetic variability and/or inbreeding depression may be detrimental for populations. Increased inbreeding leads to lower individual fitness. Loss of genetic diversity reduces the adaptive potential and might therefore reduce fitness if the environment changes. This could thus affect the potential to disperse and adapt to new environmental changes. Many exotic invasive species avoid genetic diversity loss (due to founder effects and bottlenecks) or its detrimental effects thanks to high propagule pressure (e.g. multiple independent introductions from various origins), asexuality and/or polyploidy (Roman and Darling 2007). The otter recolonisation process resembles the invasion process of several invasive species that have high colonisation success thanks to genetic admixture (Frankham 2005; Sakai et al. 2001). For example, the invasive American mink has formed feral populations in several European regions during the last century (Michalska-Parda et al. 2009; Bifolchi et al. 2010; Zalewski et al. 2010; 2011; Léger et al. 2018). Several introduction points (related to fur farms) with distinct genetic backgrounds were identified and these distinct genetic clusters have been mixing, leading to new genetic resources for these successful invasive populations.

Many native endangered species (e.g. mammals) tend to recover thanks to legal protection, a high dispersal capacity and ecological corridors. For conservation, measures to enhance genetic connectivity are considered of upmost importance to counteract inbreeding depression, preserve the evolutionary potential and ensure the long-term survival of endangered species (Edmands 2007; Spielman et al. 2004). In the studied area, connectivity might no longer be of major concern for the otter thanks to its mobility capacities. The lack of good water quality, sufficient fish resources and the availability of quiet zones might be the current limiting factors for the otter recolonisation process. Indeed, food

resources are also of upmost importance for specialist predators such as the otter. For the successful conservation of this emblematic species in France, as in the rest of Europe, special support should thus be supplied to provide suitable habitats and reasonable food resources. Freshwater habitat restoration and water pollution reductions are still critical for the recovery of otter populations (Marcelli et al. 2012). Efforts should nevertheless be maintained to preserve connectivity: first to limit and decrease road kills linked to the mobility and current recolonisation of the species (e.g. Koelewijn et al. 2010), and second to reinforce small, relict or geographically isolated populations that might benefit from gene flow from nearby populations. Finally, several complex and expensive otter reintroduction programs have yielded mixed results with poor success both in France (Alsace) and Switzerland (Mercier 2004), but more satisfactory in the Netherlands (Koelewijn et al. 2010). Conversely, the natural recovery process in France seems more efficient and appears therefore, the best option for the long term survival of this species.

Acknowledgements The authors thank all sample contributors from the different regions, and especially Sébastien Gautier (ONCFS), Ludovic Fleury and the following partners: Cistude-Nature, Charente-Nature, CEN Midi-Pyrénées, GREGE, ONCFS (a.o. Délégation Interrégionale Nord Ouest and Cellule Technique Sud Ouest), GMB, GMN, GMHL, LPO, Museum of Toulouse (MHNT), Fédération Aude Claire, Government of Navarra via the GANASA (Gestión Ambiental de Navarra), French ministry MTES and the DREAL's, SFEPM and PNA Loutre. The authors thank the editor and three anonymous reviewers for their fruitful comments on the manuscript. We are also grateful to Prof. K. Van Doninck (University of Namur) for help in fundraising and logistic support.

Author contributions CFC, DM, PF, NS, JRM and LMP designed research, performed analyses and wrote the paper. CFC, PF, DM, GC, XG, BL, FS, ES, JS and FUM contributed samples and helped for interpreting data and improving the manuscript.

Funding LMP and JRM benefited from FRS-FNRS grants ("chargée de recherches" and "directeur de recherches", respectively).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmøller MS, Enjalbert J, de Vallavieille-Pope C (2014) Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen Puccinia striiformis f.sp. tritici. PLoS One 10:e1003903

Arbos P, Meynier A (1936) Géographie du Massif Central. Revue de Géogr Alp 24(2):435–442

Awiti AO (2011) Biological diversity and resilience: lessons from the recovery of cichlid species in Lake Victoria. Ecol Soc 16(1):9

Bifolchi A, Picard D, Lemaire C, Cormier JP, Pagano A (2010) Evidence of admixture between differentiated genetic pools at a regional scale in an invasive carnivore. Conserv Genet 11:1–9

Bonesi L, Hale M, Macdonald DW (2013) Lessons from the use of non-invasive genetic sampling as a way to estimate Eurasian otter population size and sex ratio. Acta Theriol 58(2):157–168

Bouchardy C (1986) La loutre. Eds Le sang de la terre, Paris, p 174

Bouchardy C, Lemarchand C, Boulade Y, Gouilloux N, Rosoux R, Berny P (2011) Natural recolonisation of the Eurasian Otter (*Lutra lutra*) in Massif Central (France). Proc XIth Int Otter Colloquium 28B:26–29

Bryja J, Smith C, Konečný A, Reichard M (2010) Range-wide population genetic structure of the European bitterling (Rhodeus amarus) based on microsatellite and mitochondrial DNA analysis. Mol Ecol 19:4708–4722

Cabria MT, González EG, Gómez-Moliner BJ, Michaux JR, Skumatov D, Kranz A, Fournier P, Palazón S, Zardoya R (2015) Patterns of genetic variation in the endangered European mink (Mustela lutreola, 1761). BMC Evol Biol 15:141. https://doi.org/10.1186/s12862-015-0427-9

Casas-Marce M, Soriano L, López-Bao JV, Godoy JA (2013) Genetics at the verge of extinction: insights from the Iberian lynx. Mol Ecol 22:5503–5515

Chapron G, Kaczensky P, Linnell JD, Von Arx M, Huber D, Andrén H et al (2014) Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346(6216):1517–1519

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Cornuet JM, Ravigné V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v10). BMC Bioinf 11:401

Cornuet J-M, Pudlo P, Veyssier J, Dehne-Garcia A, Gautier M, Leblois R, Marin JM, Estoup A (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics 30(8):1187–1189

Dallas JF (1992) Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mamm Genome 3:452–456

Dallas JF, Piertney SB (1998) Microsatellite primers for the Eurasian otter. Mol Ecol 9:1248–1251

Dallas JF, Bacon PJ, Carss DN, Conroy JWH, Green R, Jefferies DJ, Kruuk H, Marshall F, Piertney SB, Racey PA (1999) Genetic diversity in the Eurasian otter, *Lutra lutra*, in Scotland. Evidence from microsatellite polymorphism. Biol J Linn Soc Lond 68:73–86

Dallas JF, Marshall F, Piertney SB, Bacon PJ, Racey PA (2002) Spatially restricted gene flow and reduced microsatellite polymorphism in the Eurasian otter Lutra lutra in Britain. Genetics 3(1):15–28

Demenou BB, Pineiro RP, Hardy OJ (2016) Origin and history of the Dahomey Gap separating West and Central African rain forests: insights from the phylogeography of the legume tree *Distemonanthus benthamianus*. J Biogeogr 43(5):1020–1031

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genetics Resour 4(2):359–361

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Mol Ecol 16(3):463–475

Ellegren H (1995) Mutation rates at porcine microsatellite loci. Mamm Genome 6:376–377
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
Ferrando A, Ponsà M, Marmi J, Domingo-Roura X (2004) Eurasian Otters, *Lutra lutra*, have a dominant mtDNA haplotype from the Iberian Peninsula to Scandinavia. J Hered 95(5):430–435
Frankham R (2005) Resolving the genetic paradox in invasive species. Heredity 94(4):385
Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol 24(11):2610–2618. https://doi.org/10.1111/mec.13139
Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. Conserv Biol 25:465–475
Green J, Green R (1981) The Otter (*Lutra lutra* L.) in western France. Mamm Rev 11:181–187
Griffiths HI, Thomas DH (1997) The conservation and management of the European badger (*Meles meles*). Counc Eur Nat Environ 90:86
Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806
Janssens X, Michaux JR, Fontaine MC, Libois R, de Kermabon J, Defourny P, Baret PV (2008) Non-invasive genetics at basin scale reveals barriers to the present otter recovery in Southern France. Ecography 31:176–186
Jefferies DJ (1989) The changing otter population in Britain 1700–1989. Biol J Linn Soc Lond 38:61–69
Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27(21):3070–3071
Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94
Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol 4:782–788
Koelewijn HP, Pérez-Haro M, Jansman HAH, Boerwinkel MC, Bovenschen J, Lammertsma DR, Niewold FJJ, Kuiters AT (2010) The reintroduction of the Eurasian otter (*Lutra lutra*) into the Netherlands: hidden life revealed by noninvasive genetic monitoring. Conserv Genet 11:601–614
Kolbe JJ, Glor RE, Rodríguez Schettino L, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181
Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15(5):1179–1191
Kruuk H (2006) Otters: ecology, behaviour and conservation. Oxford Universtiy Press, Oxford. https://doi.org/10.1093/acprof:oso/9780198565871.001.0001
Kuhn R (2011) La Loutre d'Europe. In: Artois M, Haffner P, Jacques H, Moutou F, Maurin H (eds) Encyclopédie des carnivores de France. SFEPM, Bourges
Langella O (2007) Populations 1.2.30: Population genetic software (individuals or populations distances, phylogenetic trees). http://bioinformatics.org/~tryphon/populations/. Accessed 12 May 2015
Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104:3883–3888
Leblanc F, Dohogne R, Guischer V, Thuaire N, Bellanger C, Constant J (2005) Actualisation de la répartition de la Loutre d'Europe en Limousin (2003/2004). La Conservation de la Loutre, actes du XXVIIème Colloque Francophone de Mammalogie de la SFEPM, pp 9-28.
Legendre P, Legendre LFJ (1998) Numerical ecology. Elsevier Science, 852 pp
Léger F, Steinmetz J, Laoué E, Maillard J-F, Ruette S (2018) L'expansion du vison d'Amérique en France. Période 2000–2015. Faune Sauvage 138:23–31
Li YL, Liu JX (2018) StructureSelector: a web based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176–177
Maran T, Skumatov D, Gomez A, Põdra M, Abramov AV, Dinets V (2016) *Mustela lutreola*. The IUCN Red List of Threatened Species 2016: e.T14018A45199861
Marcelli M, Polednik L, Polednikova K, Fusillo R (2012) Land use drivers of species re-expansion: inferring colonization dynamics in Eurasian otters. Divers Distrib 10:1001–1012
Mathias P (1933) Sur la répartition de la Loutre en France. Bull de la Soc centrale d'Aquicult et de pêche 40:73–78
Matocq M, Villablanca F (2001) Low genetic diversity in an endangered species: recent or historical pattern? Biol Cons 98(1):61–68
Michalska-Parda A, Brzezinski M, Zalewski A, Kozakiewicz M (2009) Genetic variability of feral and ranch American mink *Neovison vison* in Poland. Acta Theriol 54:1–10
Mercier L (2004) Bilan de la réintroduction de la Loutre *Lutra lutra* (Linné, 1758) en Alsace, France. Bull de la Soc d'histoire naturelle de Colmar 65:117–134
Michaux JR, Hardy OJ, Justy F, Fournier P, Kranz A, Cabria M, Davison A, Rosoux R, Libois R (2005) Conservation genetics and population history of the threatened European mink Mustela lutreola, with an emphasis on the west European population. Mol Ecol 14:2373–2388
Mondol S, Bruford MW, Ramakrishnan U (2013) Demographic loss, genetic structure and the conservation implications for Indian tigers. Proc Royal Soc B 280:20130496
Mucci N, Arrendal J, Ansorge H, Bailey M, Bodner M, Delibes M, Ferrando A, Fournier P, Fournier C, Godoy JA, Hajkova P, Hauer S, Heggberget TM, Heidecke D, Kirjavainen H, Krueger H-H, Kvaloy K, Lafontaine L, Lanszki J, Lemarchand C, Liukko U-M, Loeschcke V, Ludwig G, Madsen AB, Mercier L, Ozolins J, Paunovic M, Pertoldi C, Piriz A, Prigioni C, Santos-Reis M, Luis TS, Stjernberg T, Schmid H, Suchentrunk F, Teubner J, Tornberg R, Zinke O, Randi E (2010) Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. Conserv Genet 11:583–599
Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 19:153–170
Ogutu-Ohwayo R (1990) The decline in the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced fishes, especially Nile perch, *Lates niloticus* and Nile tilapia *Oreochromis niloticus*. Environ Biol Fishes 27:81–96
Olafsson K, Pampoulie C, Hjorleifsdottir S, Gudjonsson S, Hreggvidsson GO (2014) Present-day genetic structure of Atlantic salmon (Salmo salar) in Icelandic rivers and ice-cap retreat models. PLoS ONE 9:e86809
Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol ecol 4(3):347–354
Palstra FP, Fraser DJ (2012) Effective/census population size ratio estimation: a compendium and appraisal. Ecol Evol 2:2357–2365
Pannell JR, Charlesworth B (1999) Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonisation. Evolution 53:664–676
Penez J (2004) Les réseaux d'investissement dans le thermalisme au XIXe siècle en France. In Situ [En ligne], 4, online on 1st March 2004, consulted on 20th April 2019

Pérez-Haro M, Vinas J, Manas F, Batet A, Ruiz-Olmo J, Pla C (2005) Genetic variability in the complete mitochondrial control region of the Eurasian Otter (*Lutra lutra*) in the Iberian Peninsula. Biol J Linn Soc 86(4):397–403

Pigneur L-M, Etoundi E, Aldridge DC, Marescaux J, Yasuda N, Van Doninck K (2014) Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. Mol Ecol 23(20):5102–5116

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Heredity 95(6):536–539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Puechmaille SJ (2016) The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16(3):608–627

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org. Accessed 20 July 2015

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Randi E, Davoli F, Pierpaoli M, Pertoldi C, Madsen AB, Loeschcke V (2003) Genetic structure in otter (*Lutra lutra*) populations in Europe: implications for conservation. Anim Conserv 6:1–10

Reuther C, Krekemeyer A (2004) On the way towards an Otter Habitat Network Europe (OHNE). Method and results of an assessment on the European and the German level. Habitat 15:308

Rice WR (1989) Analyzing tales of statistical tests. Evolution 43:223–225

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Saavedra D, Sargatal J (1997) Reintroduction of the otter (*Lutra lutra*) in northeast Spain (Girona province). Galemys 10:191–199

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. Theor Popul Biol 12:253–262

Spielman D, Brook BW, Briscoe DA, Frankham R (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? Conserv Genet 5(4):439–448

Stanton DWG, Hobbs GI, McCafferty DJ, Chadwick EA, Philbey AW, Saccheri IJ, Slater FM, Bruford MW (2014) Contrasting genetic structure of the Eurasian otter (*Lutra lutra*) across a latitudinal divide. J Mamm 95(4):814–823

Steinmetz J, Marc D, Néri F, Trichet E, Besnard A, Defos du Rau P, Bodin J (2014) Dynamique régionale de la loutre en Midi-Pyrénées. Faune Sauv 305:31–37

Sunnaker M, Busetto AG, Numminen E, Corander J, Foll M, Dessimoz C (2013) Approximate bayesian computation. PLoS Comput Biol 9:e1002803

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389–399

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. Proc Natl Acad Sci USA 97:5948–5953

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 10:1471–1478

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123–1128

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. Trends Ecol Evol 30(1):42–49

Witte F, Msuku BS, Wanink JH, Seehausen O, Katunzi EFB, Goudswaard PC, Goldschmidt T (2000) Recovery of cichlid species in Lake Victoria: an examination of factors leading to differential extinction. Rev Fish Biol Fish 10:233–241

Zalewski A, Michalska-Parda A, Bartoszewicz M, Kozakiewicz M, Brzeziński M (2010) Multiple introductions determine the genetic structure of an invasive species population: American mink *Neovison vison* in Poland. Biol Conserv 143(6):1355–1363

Zalewski A, Michalska-Parda A, Ratkiewicz M, Kozakiewicz M, Bartoszewicz M, Brzeziński M (2011) High mitochondrial DNA diversity of an introduced alien carnivore: comparison of feral and ranch American mink *Neovison vison* in Poland. Divers Distrib 17:757–768

Zhang Y-Y, Zhang D-Y, Barrett SCH (2010) Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Mol Ecol 19:1774–1786

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.